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Changes in the molecular weight distribution of soil organic matter during humification

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Abstract Changes in the molecular weight distribution of soil organic matter during humification were studied by incubating soil samples over a period of up to 600 days with and without the addition of a broadleaf forest and *Pinus radiata* litter. With prolonged incubation both kinds of litter showed a marked continuous decrease of organic matter in the high molecular weight (>100 000) range. On the other hand, the soil incubated without litter addition showed an increase of the >100 000 molecular weight fraction with time. These observations support previous results on changes in the molecular weight distribution of soil organic matter during soil development in a chronosequence. They are also consistent with the changes in the molecular weight of organic matter on forest floors.

Keywords Soil chemistry; litter; humus; molecular weights; distributions; changes; forest soils; soil sequences.

INTRODUCTION

Changes in the molecular weight distribution of soil organic matter during humification have been reported by several workers (e.g., Swift et al. 1970; Aleksandrova 1972; Kumada & Miyara 1973; Fokin et al. 1974; Hayes et al. 1975). However, most of these studies involved measurements of molecular weight changes of fresh plant residues and humic extracts added to soils in the laboratory. There is no reported work which examined the changes in the molecular weight distribution of organic matter in soils during humification. Changes in the molecular weight distribution of extracted fractions of forest litter before and after addition to the soil in incubation experiments have also not been studied.

The main objective of the present study is to gain some insight into changes in the molecular weight distribution of soil organic matter during humification. These results are then compared with those reported earlier for field soil samples representing members of different soil chronosequences (Goh & Williams 1979).

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MATERIALS AND METHODS

Freshly collected field soil samples were incubated in the laboratory either alone or with the addition of their own forest litter or that from the exotic forest plantation (*Pinus radiata*). Over a period of up to 600 days, incubated soil and litter samples were extracted periodically, and the nominal molecular weight distribution of purified organic matter extracts was determined using the gel filtration technique (Goh & Reid 1975).

Soil and litter used

The A horizon of the Koputaroa soil from the Manawatu chronosequence was used. Some properties of this soil and its relationship to other members of the Manawatu chronosequence and of other chronosequences have been published (Goh et al. 1976; Goh & Williams 1979). Before use, the soil was passed through a 2 mm sieve.

The forest litter was collected from the Koputaroa site where the soil was sampled. *Beilschmiedia tawa* and *Melicytus ramiflorus* are the main tree species in this coastal broadleaf forest. Pine (*Pinus radiata*) needles were obtained from Granville Forest in the Grey valley of Westland, New Zealand. All litter was air dried and then crushed finely by hand.

Incubation procedure

The treatments included: soil alone (20 g Koputaroa soil) as control; 2 g of either Koputaroa forest litter or pine needles placed on top of the soil without mixing; 2 g of litter mixed thoroughly with the soil using a spatula. Each sample was placed in a small glass jar, and water was added to bring the moisture content to 60% of the water-holding capacity of the soil.

These jars were placed in a transparent plastic tank and partly covered with a transparent plastic lid with sufficient gaps to allow air to circulate. Water was placed in the bottom of the tank so as to maintain a moist atmosphere. Since room temperature was recommended (A. P. Mulcock, pers.

Table 1 Molecular weight distribution (%) of organic matter extracted from the soil with and without the addition of Koputaroa forest litter at different times of incubation.

Duration of incubation (days)	Extractant and estimate	Soil alone				Litter on soil surface				Litter mixed with soil			
		MW1†	MW2	MW3	MW4	MW1	MW2	MW3	MW4	MW1	MW2	MW3	MW4
0	Pyrophosphate	65.0	8.0	12.0	15.0	97.0	0	3.0	n.d.	70.0	11.0	5.0	14.0
	0.5*M* NaOH	84.0	7.0	4.0	5.0	89.0	5.0	6.0	n.d.	90.0	0	10.0	n.d.
	Weighted mean distribution	75.2	7.5	7.7	9.6	90.3	4.3	5.5	n.d.	78.9	6.1	7.2	7.8
29	Pyrophosphate	69.0	3.0	6.0	22.0	79.0	11.0	10.0	0	70.5	4.5	9.0	16.0
	0.5*M* NaOH	83.0	7.0	4.0	6.0	92.0	0	8.0	n.d.	83.0	7.0	3.0	7.0
	Weighted mean distribution	77.0	5.3	4.9	12.8	90.3	1.4	8.3	n.d.	78.7	6.1	5.0	10.2
124	Pyrophosphate	55.0	10.0	11.0	24.0	76.0	4.0	6.0	14.0	73.0	1.0	5.0	21.0
	0.5*M* NaOH	86.0	0	2.0	12.0	73.0	0	20.0	7.0	85.0	0	4.0	11.0
	Weighted mean distribution	72.0	5.0	6.0	17.0	73.6	0.8	17.1	8.4	79.7	0.4	4.5	15.4
319	Pyrophosphate	66.6	6.7	2.8	23.9	89.7	0	10.3	n.d.	77.2	0.8	2.5	19.6
	0.5*M* NaOH	83.5	1.0	7.8	7.8	76.2	0	4.7	19.1	85.9	0	1.6	12.5
	Weighted mean distribution	75.5	3.7	5.4	15.5	78.7	0	5.7	15.6	82.5	0.3	2.0	15.2
600	Pyrophosphate	70.7	0	8.2	21.2	n.d.	n.d.	n.d.	n.d.	70.5	0.4	5.6	23.6
	0.5*M* NaOH	91.3	0	2.9	5.8	n.d.	n.d.	n.d.	n.d.	93.7	0	6.3	0
	Weighted mean distribution	81.9	0	5.3	12.9	n.d.	n.d.	n.d.	n.d.	84.6	0.1	6.1	9.2

†Molecular weight distribution: MW1 = >100 000; MW2 = 100 000–50 000; MW3 = 50 000–10 000; MW4 = <10 000.
n.d., not determined.

comm.) as the most suitable temperature for the incubation of soil, the tank and contents were placed on a bench in the laboratory.

Each week, the temperature of the tank was recorded from a thermometer placed in the water in the tank, and the weight of each jar was adjusted so as to maintain the moisture content. Over 600 days the temperature showed a mean of 19.7°C and a range of 22.3–16.4°C.

Two jars from each set of treatments were taken out of the tank after 0, 29, 124, 319, and 600 days of incubation. The pine litter treatment was terminated after 319 days. The 2 samples were combined, air dried, and ground to pass through a 0.16 mm sieve. Tweezers were used to remove the litter from the soil surface.

Extraction, purification, and fractionation of organic matter

The methods used have been described previously (Goh & Reid 1975; Goh & Williams 1979). After acid pre-treatment (0.1*M* HCl followed by 0.1*M* HCl–0.3*M* HF) the soil samples were extracted with 0.1*M* $Na_4P_2O_7$ (pH 7.0) followed by 0.5*M* NaOH. Extracts were purified and fractionated into 4 nominal molecular weight ranges (>100 000; 100 000–50 000; 50 000–10 000; <10 000) using gel filtration. Some of the 0.1*M* $Na_4P_2O_7$ extracts were further fractionated into humic and fulvic acids according to Kononova (1966). The carbon content of the purified extracts was determined in duplicate using the Walkley and Black method (Allison 1965). The fulvic acid carbon was obtained by difference.

RESULTS

Soil alone

About 75% of the extracted organic matter in the soil was in the >100 000 molecular weight range (Table 1). In the initial stages (29, 124 days), the weighted mean molecular weight distribution of soil organic matter showed little change, but at 600 days the proportion of organic matter in the >100 000 molecular weight range increased markedly. This trend was also shown by the organic matter in the individual extracts, although some large changes occurred in the pyrophosphate extract at 124 days, and in the NaOH extract at 600 days. No visible colour changes were observed in the organic matter extracts.

Most of the changes in the carbon content of humic components were shown by the fulvic acid fraction (Fig. 1) which increased in the early part of

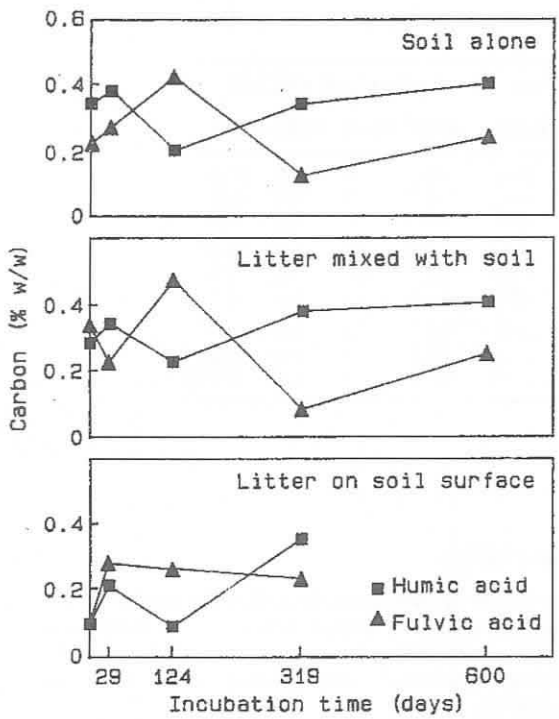

Fig. 1 Distribution of carbon (% w/w) in humic and fulvic acid in the pyrophosphate (0.1*M* $Na_4P_2O_7$) extract of soil with and without the addition of Koputaroa forest litter at different times of incubation.

incubation period (29 and 124 days). By 600 days the distributions of humic and fulvic acids had become very similar to that in the soil before incubation. This suggests that steady state conditions have been attained.

Incubation of Koputaroa forest litter

Litter on soil surface

At zero time, 90% of the extracted organic matter was in the >100 000 weighted mean molecular weight range, being higher in the pyrophosphate than in the subsequent NaOH extract (Table 1). After 29 days of incubation, this fraction remained the same, although the proportion in the <50 000 molecular weight range increased. The latter was due to a marked reduction in the >100 000 molecular weight fraction of the pyrophosphate extract.

Further changes in the weighted mean molecular weight distribution occurred at 124 days. With prolonged incubation (319 days) 79% of the organic matter occurred in the >100 000 molecular weight range. Thus the humification of litter resulted in a reduction in the molecular weight of organic matter.

Colour changes in the extracted organic matter from the decomposed litter were observed during incubation. It was very pale yellow before incubation, but changed to a brownish colour by 124 days. This shows that colour changes of decomposed litter are related to changes in molecular sizes of soil organic matter (Swift et al. 1970). Colour changes in decomposed plant residues during the humification of various fresh plant residues were reported by Kononova (1966).

The humic components showed similar changes to those observed for the soil alone treatment (Fig. 1). An increase in the fulvic acid fraction occurred in the early part of the incubation period (29 days). This is similar to that found by Aleksandrova (1972) during the humification of plant residues in loam. However, the humic acid fraction became more important at 319 days.

Litter mixed with soil

At zero time, nearly 80% of the organic matter has a weighted mean molecular weight >100 000, this being higher in the NaOH extract (Table 1). During incubation, changes in molecular weight distribution were slight with a tendency towards the >100 000 molecular weight range. Some small changes in the individual extracts also occurred in the initial incubation period (29, 124, 319 days). However, at 600 days the molecular weight distribution appeared to be similar to that of the sample before incubation, suggesting that an apparent steady state condition had been reached. No obvious colour changes of soil organic matter extracts were observed.

The proportions of humic acid and fulvic acids changed markedly with time of incubation (Fig. 1). However, there was no consistent trend although the distribution became similar to that of the soil alone before and after prolonged incubation (600 days). This suggests that the effect of mixing the litter with the soil on litter decomposition becomes undetectable after 600 days, when results observed were due entirely to the organic matter present in the soil alone treatment.

Incubation of *Pinus radiata* litter

Litter on soil surface

After 319 days of incubation, the amount of organic matter in the >100 000 molecular weight range decreased from about 89 to 67% (Table 2). The decrease was greater than that shown by Koputaroa forest litter on the soil surface (Table 1). Changes in the molecular weight distribution of organic matter in the pyrophosphate and subsequent NaOH extracts were somewhat similar to those observed for Koputaroa forest litter. However, at 319 days

Table 2 Molecular weight distribution (%) of organic matter extracts during incubation of *Pinus radiata* litter.

Duration of incubation (days)	Extractant and estimate	Litter on soil surface				Litter mixed with soil			
		MW1†	MW2	MW3	MW4	MW1	MW2	MW3	MW4
0	Pyrophosphate	92.4	0	7.6	n.d.	72.1	0	0.6	27.3
	0.5*M* NaOH	88.8	0	11.2	n.d.	92.2	0	7.8	n.d.
	Weighted mean distribution	88.9	0	11.1	n.d.	85.3	0	5.3	9.4
124	Pyrophosphate	82.9	10.2	7.0	n.d.	78.6	3.8	2.7	14.8
	0.5*M* NaOH	80.5	3.5	3.4	12.6	83.7	9.0	0	7.3
	Weighted mean distribution	80.6	3.8	3.6	12.0	82.5	7.8	0.7	9.1
319	Pyrophosphate	100.0	0	0	n.d.	72.3	0	9.7	18.0
	0.5*M* NaOH	67.0	0	33.0	0	94.7	0	4.4	0.9
	Weighted mean distribution	67.4	0	32.6	0	87.1	0	6.2	6.7

†For explanation of terms see Table 1.

the pyrophospate extract showed a very low carbon content (0.06% C compared to 0.22% C at 124 days of incubation). Practically all the organic matter in this extract was in the >100 000 molecular weight fraction, suggesting the presence in the litter of a residue which was slow to undergo microbial decomposition. Furthermore, the pyrophosphate extract remained pale in colour throughout the incubation, whereas the subsequent NaOH extract was very dark and viscous. Kononova (1966) reported that Scots pine needles were slow to humify and attributed this to the high lignin and cellulose content and very low protein (N) content of the unhumified compounds.

Litter mixed with soil

The results (Table 2) showed very little change in the molecular weight distribution of soil organic matter when pine needle litter was incorporated into the soil before incubation. The same tendency was observed when Koputaroa forest litter was used (Table 1).

Comparisons of surface and mixed litter

Results of comparisons between the Koputaroa forest litter and the pine needles (Table 3) showed that when the litter was placed on the soil surface, changes in the molecular weight distribution of litter during incubation were more pronounced than when it was mixed with the soil prior to incubation. In the natural forest floor, litter usually accumulates on the soil surface. Thus the results obtained when the litter was placed on the soil surface are more representative of natural forest conditions than those from mixing litter with the soil prior to incubation.

DISCUSSION

The results of this study show that about 90% of the organic matter in forest litters has a molecular weight >100 000. This high molecular weight fraction decreased continuously with time of incubation.

Laboratory incubation studies, using various fresh plant residues, have shown that the molecular weight of organic matter increased with time. For example, Bloomfield et al. (1975) observed an increase in the molecular weight of water-soluble extracts from <1000 to >30 000 during the aerobic incubation of fresh lucerne leaves as well as a progressive darkening of the extracts from dark brown to a golden yellow colour. However, these workers probably measured an earlier stage of humification than that observed here. The forest litters in the present study were brown in colour and were probably already partly humified prior to collection. Kononova (1966) has given evidence for humification in oak, hazel, and birch leaves before their lignified tissues decomposed.

Our results suggest that the initial microbial breakdown of fresh plant residues is followed by the synthesis of newly formed brownish humic substances of high molecular weight. The latter process could occur in the litter during senescence and before it is completely incorporated into the soil. Kononova (1966) showed that changes in the chemical composition of plant residues occurred at different rates depending on the chemical composition of the residues, particularly the relative proportions of compounds such as hemicelluloses, cellulose, lignin, protein, and ethanol–benzene soluble compounds.

Changes in the molecular weight distribution of organic matter during the incubation of *Pinus*

Table 3 Comparison of molecular weight distribution of organic matter extracts from different treatments of Koputaroa forest and *Pinus radiata* litter during incubation.

Treatment†	Duration of incubation (days)	Pyrophosphate				0.5*M* NaOH				Weighted mean distribution			
		MW1	MW2	MW3	MW4	MW1	MW2	MW3	MW4	MW1	MW2	MW3	MW4
Koputaroa													
L−S	0	32.0	−8.0	−24.0	n.d.	5.0	−2.0	−3.0	n.d.	15.0	−3.2	11.8	n.d.
	29	10.0	8.0	−18.0	n.d.	9.0	−7.0	−2.0	n.d.	13.3	−3.9	−9.4	n.d.
	124	21.0	−6.0	−5.0	−10.0	−13.0	0	18.0	−5.0	1.3	−3.6	11.2	−8.9
	319	23.0	−6.7	n.d.	−16.4	−7.3	−1.0	−3.0	11.4	3.2	−3.7	0.3	0.2
(L+S)−S	0	5.0	3.0	−7.0	−1.0	6.0	−7.0	1.0	n.d.	3.6	−1.3	−0.5	−1.8
	29	1.5	1.5	3.0	−6.0	0	0	−1.0	1.0	1.6	0.9	0.2	−2.7
	124	18.0	−9.0	−6.0	−3.0	−1.0	0	2.0	−1.0	7.4	−4.0	−1.5	−1.9
	319	10.6	−5.9	−0.4	−4.3	2.4	−1.0	−6.1	4.7	7.1	−3.4	−3.4	−0.2
Pinus radiata													
L−S	0	27.4	−8.0	−20.6	n.d.	4.8	−7.0	2.2	n.d.	13.0	−7.5	−6.3	n.d.
	124	27.9	0.2	−28.1	n.d.	−5.5	3.5	1.4	0.4	8.3	−0.6	−2.4	−5.3
	319	33.4	−6.7	−26.7	n.d.	−16.5	−1.0	25.5	−7.8	−8.0	−3.7	27.2	−15.5
(L+S)−S	0	7.1	−8.0	−11.4	12.3	8.2	−7.0	−1.2	n.d.	10.1	−7.5	−2.4	−0.2
	124	23.6	−6.2	−8.3	−9.2	2.3	9.0	−2.0	−4.7	10.2	3.3	−5.3	−8.2
	319	5.7	−6.7	6.9	−5.9	11.2	−1.0	−3.4	−6.9	11.6	−3.7	0.8	−8.7

†S = soil alone; L = litter on soil surface; (L+S) = litter mixed with soil. Negative (−) difference implies higher amount in S than L or (L+S). For explanation of other terms see Table 1.

radiata litter were not as evident as that observed for Koputaroa forest litter, probably due to the different chemical nature of the litter. The higher nitrogen content of the Koputaroa forest litter (1.28% as compared with 0.82% for pine needles) would probably favour a faster rate of humification.

Soil alone, on incubation, showed a significant increase in the organic matter in the >100 000 molecular weight range by 600 days. This trend contrasts to the situation during litter humification where the molecular weight decreased with time of incubation. Forest litter, when added to soil, apparently breaks down to smaller molecules which recombine to form larger molecules during humification. In the A horizons of soils under natural conditions, both processes probably occur together, but at different rates. Depending on soil biological activity and environmental conditions either a net accumulation or a complete incorporation of litter into soil organic matter, or both, would be favoured.

In the soils of the Manawatu chronosequences (see Goh et al. 1976) the A horizon of Waitarere soil does not accumulate a very distinct litter layer (Cowie 1968) probably due to the rapid breakdown of plant residue (Stout & Dutch 1968). Thus the humification of soil organic matter would be the dominant process in this soil (estimated age = <100 years). This would favour the formation of high molecular weight materials (see Goh & Williams 1979). However, in the older soils such as Koputaroa (estimated age = 10 000–15 000 years), where litter tends to accumulate in the topsoils, litter breakdown would be expected to give rise predominantly to low molecular weight materials. The process, however, would occur simultaneously with humification of soil organic matter which favours the formation of high molecular weight materials. Furthermore, any litter which accumulates on the soil surface is likely to be partly humified and hence contains a predominance of high molecular weight materials. The net result is an increase with time of organic matter with intermediate molecular weight (Goh & Williams 1979).

In the very old, highly weathered and strongly leached Okarito soil (⩾22 000 years; see Tan 1979), the acidic and extremely wet conditions prevent rapid humification and incorporation of plant residues. This would lead to the accumulation of a greater proportion of high molecular weight material with very little material in the other molecular weight ranges. This postulate is supported by the earlier results of Goh & Williams (1979).

As far as we are aware, there is no reported work on the changes in the molecular weight of organic matter extracted from litter and soil during incubation, although results have been reported for humic materials and fresh plant residues. For example, Aleksandrova (1972) found the molecular weight distribution of newly formed humic acids remained high during the entire period of

humification of plant residues in soil. This led her to propose that humic acids apparently formed from high molecular weight decomposition products of plant and microbial residues rather than from 'monomers' that gradually condensed into large molecules, and their molecular weights gradually decreased during humification. Aleksandrova (1972) also found that more fulvic acid than humic acid was formed on incubation of clover leaves in soil for 1 month.

CONCLUSIONS

Prolonged incubation of litter (319 days) on the soil surface resulted in a marked decrease in the amount of organic matter in the >100 000 molecular weight range—from 90 to 79%. On the other hand, on incubating the soil alone for 600 days, the organic matter showed a marked increase in the >100 000 molecular weight range. However, the incubation of litter mixed with soil caused only slight changes in the molecular weight distribution of the organic matter. After 600 days this distribution appeared to be the same as that before incubation, suggesting the attainment of steady state conditions.

Although the maximum period of incubation in the present study is only 600 days, the results appear to support earlier observations on changes in the molecular weight distribution of soil organic matter during soil development (Goh & Williams 1979). The incubation results indicate that changes in the molecular weight distribution of organic matter during humification of litter (litter on soil surface) are different from those observed during soil organic matter humification (soil alone). The former process leads to a predominance of low molecular weight material while the latter results in high molecular weight materials. Under natural conditions, the molecular weight distribution of organic matter reflects the net effect of these 2 processes. In the very young Waitarere soil, for example, where litter accumulation is almost absent, the molecular weight of organic matter is expected to be high. In contrast, in older soils (e.g., Koputaroa) where litter accumulation occurs, intermediate molecular weight material becomes important. In the very old soil (e.g., Okarito) where soil conditions prevent rapid humification and incorporation of plant residues, accumulation of high molecular weight material occurs with very little material in the low molecular weight range.

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